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Putting hydrodynamic interactions to work: tagged particle separation

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Abstract. – Separation of magnetically tagged cells is performed by attaching markers to a subset of cells in suspension and applying fields to pull from them in a variety of ways. The magnetic force is proportional to the field *gradient*, and the hydrodynamic interactions play only a passive, adverse role. We propose using a homogeneous rotating magnetic field only to make tagged particles rotate, and then performing the actual separation by means of hydrodynamic interactions, which thus play an active role. The method, which we explore here theoretically and by means of numerical simulations, lends itself naturally to sorting on large scales.

The appearance of immunomagnetic beads — superparamagnetic nano or micro-particles attached to an antibody — increased considerably the possibilities for cell detection and isolation. Suitable antibodies can be chosen to bind the beads to the desired cells, which are then magnetically marked, leading to a very rich tool for separation.

Immunomagnetic separation has become very popular in the last years. However, there has been more progress in the quality of beads themselves than in the processing methods [1]. (For details on existing techniques, see, for example, [2, 3]). In the usual situation, normal and tagged cells are in suspension in a liquid and, in all the existing separation techniques, magnetic field *gradients* are used to drag the latter through the liquid. The need to maximize gradients in order to improve efficiency imposes reduced geometries and hence introduces practical limitations which may turn out to be important in applications with very large total number of cells. This can in principle be remedied using stronger magnetic fields and bead moments, but then undesired clustering due to magnetic attraction between tagged cells appears. On the other hand, cell concentrations have to be kept low to prevent normal cells from being dragged by tagged ones through hydrodynamic interactions.

The goal of this letter is to introduce a very different approach: it consists of using the magnetic field *only* to make the tagged particles spin, and to use the resulting hydrodynamic interactions to effect the separation. We test this approach numerically, the method of simulation we used is described later in this text. Forced spinning can be produced by a spatially *homogeneous* rotating magnetic field and, as we shall see, interparticle forces do not represent a drawback but actually produce the separation. We shall consider two settings: The first, used for continuous sorting of particles, is reminiscent of Free-Flow Magnetophoresis [2], although it may be implemented in a wide channel. The second is a many-body dynamical phase-separation transition, in itself and intriguing phenomenon that to our knowledge has not been described before.

Let us first discuss why it is advantageous to use fields to *spin*, rather than to *displace* the particles. Consider a spherical particle of radius a and magnetic dipole moment m immersed in a fluid of viscosity η . We can displace the particle by applying a static field Fig. 1 (left), in which case the force is proportional to the field's *gradient*. Alternatively, we can apply a rotating field (right), and the torque and angular velocity will be proportional to the field *amplitude*. In order to convert rotation into translation we need an extra force: in this simple example we suppose we can force the particle to rotate without sliding on a line.

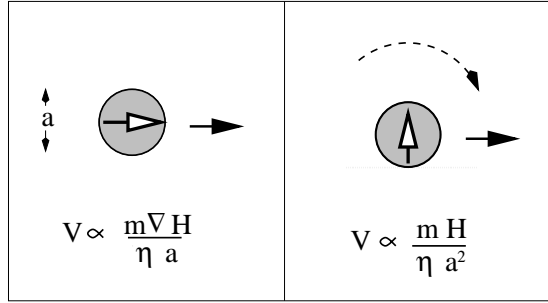


Fig. 1 – A particle being pulled by a force gradient (left), and made to roll on a rough line by a rotating field (right). The black and white arrows indicate the direction of the field and moment, respectively (both rotate in the figure on the right).

Let us compare the efficiency of each method: Applying a magnetic field H the force on the particle is $\propto m \nabla H \sim m H / \ell$, where ℓ is the typical length over which the field varies. The resulting velocity is given by Stokes' law $v_{static} \sim \frac{m H}{6 \pi \eta a \ell}$. If instead we apply a rotating field, setting the field's frequency ω to be the fastest that the particle's rotation can follow, the angle between field and magnetic moment approaches ninety degrees, and the torque τ on the particle is $\sim m H$. For an isolated particle, Stokes' law gives $\omega \sim \frac{m H}{8 \pi \eta a^3}$. Imposing that the particle rolls without sliding on the line within the liquid, the rotational motion is converted by friction into linear motion $v_{rot} = \omega a$, i.e. $v_{rot} \sim a \frac{m H}{8 \pi \eta a^3} \propto \frac{m H}{\eta a^2}$. Comparing both mechanisms, we have:

$$\frac{v_{rot}}{v_{static}} \propto \frac{H}{\nabla H a} \sim \frac{\ell}{a} \quad (1)$$

i.e. the advantage of using rotation over using a static field is in proportion to the typical range of variation of fields (e.g. the distance between magnetic poles), compared with the particle size — typically a few orders of magnitude [4].

In what follows, we will show that one can apply the same principle [5], but with the role of transforming rotational into translational motion played by the hydrodynamic interactions,

and to a lesser extent, the direct inter-particle forces. Depending on the setting, the interaction is *i*) between the rotating cells and a porous medium or a specially designed set of obstacles, *ii*) between marked and unmarked cells and *iii*) between cells and the walls.

Let us note here that the ferromagnetic particle need not have a permanent magnetic moment: the only necessary condition is that when subjected to a rotating field the angle of the magnetization direction lags with respect to that of the field (i.e. that there is hysteresis), and this will happen in a ferromagnetic substance whether permanently magnetized or not. In the latter case the estimate (1) will be modified by a hysteresis-dependent factor, but the force will still depend upon the field rather than its gradient.

Hydrodynamic mechanisms that convert rotation into lateral displacements have been known for a long time (for example, see [6, 7]): the question here is to devise a method that works at zero Reynolds number so it does not become inefficient for particle sizes and liquid viscosities involved in biological applications.

Stokesian Dynamics

Let us briefly discuss how the results in this letter were obtained. The dynamics of the particles in the suspension at zero Reynolds number was simulated by means of a Stokesian Dynamics (SD) algorithm [8, 9, 10, 11, 12, 13, 14, 15], which involves various levels of approximation. We use the so-called F-T (force-torque) version which provides excellent results when no external linear shear is imposed on the flow and diminishes the computing time by a factor ≈ 6.2 with respect to the most elaborate approximation [8]. At this level, the velocities of the N suspended particles and the forces and torques acting on them are related by a grand resistance matrix \mathcal{R}^* , $F = \mathcal{R}^*(U - \langle u \rangle)$, where the $6N$ -dimensional vectors F and U represent the applied forces-torques and the linear-angular velocities, respectively, and $\langle U \rangle$ is the average velocity of the suspension (particles and fluid). Details of the grand resistance matrix construction are given in ref [9]. Briefly, it works in two steps: The inverse of \mathcal{R}^* is first approximated by a far-field, multipole expansion. The box of containing the N particles is periodically repeated [10] and the convergence of the sum over all the long-range interactions is accelerated by the Ewald summation technique [11]. Near field contributions are finally included in a pairwise additive fashion at the level of \mathcal{R}^* using exact two-sphere resistance functions [12, 13].

In all our SD simulations, every particle has a radius a . The elementary cell containing the N suspended particles is a box of sides L_x , L_y and L_z in the directions x , y and z , respectively. The particles are always in the $x - y$ plane. Applied torques are in the z direction. The obstacles used for continuous separation are represented by immobile particles which have the same radius that the suspended ones, and similarly, the bottom wall we need for collective segregation is simulated with fixed particles of radius a (see for example in ref. [14, 15]). Finally, sedimentation, when present (see Fig. 3), is in the negative y direction.

Continuous separation.

An implementation of continuous sorting or enriching of tagged cells that works in the high viscosity limit is the following: An average flow of velocity U_d is sustained through a medium with fixed obstacles. These could for example be filaments with axis perpendicular to the flow — like the hairs of a brush. A rotating field is applied, imposing that marked particles rotate (with angular velocity ω , when isolated) around an axis parallel to the obstacles.

Particles forced to rotate are made by hydrodynamic interactions to describe arcs around the obstacles: if their density is high enough that these deflections overlap, a lateral diffusivity results (Fig. 2-(left)). On the contrary, unmarked particles will be carried by the flow, gently avoiding the obstacles (Fig. 2-(right)). A continuous enrichment method can then be

implemented by selecting through appropriate windows (at the right hand side of the channel shown in Fig. 2) either the magnetic or the non-magnetic particles.

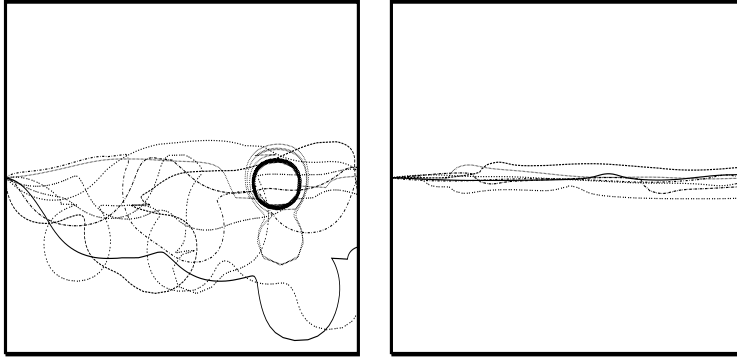


Fig. 2 – Stokesian dynamic trajectories corresponding to a particle of radius a entering the fluid on the left. Fourteen fixed obstacles, also of radius a were placed at random in the rectangle (not shown). Each curve corresponds to a different configuration of the obstacles. Left: marked particles under a torque τ perpendicular to the paper and $\omega a/U_d = 100$. Right: unmarked particles. The sides of the unit cell are $L_x = 40a$, $L_y = 40a$ and $L_z = 200a$.

Purely hydrodynamic interactions give a symmetric diffusivity on average [16]. Although unnecessary for enrichment purposes, one can also generate a net lateral drift. Marked-particle diffusivity increases with obstacle density: then, by making the latter spatially dependent, one can take advantage of the general fact that diffusive particles tend to accumulate in regions of smaller diffusivity [17].

In the usual free-flow magnetophoresis [18] a pair of wedge-type magnetic poles on the sides of a channel that necessarily has to be thin, in order to maximize the field gradient. In the method described above, the separation is proportional to the intensity of the rotating field, there is no need to have a field gradient and hence there is the possibility of working with a thicker channel, weaker fields or smaller marker moments. Let us make a rough comparison: When the deflection is caused by a stationary field, we may give a particle a lateral velocity U_{trans} . The separation will be the faster, the larger the drift velocity U_d , as this reduces the passage time $t_{trans} \sim L/U_d$, with L the typical length of the channel. However, geometry requires that particles deflect a certain angle $\tan \theta = U_{trans}/U_d$, so that $t_{trans} \sim L \tan \theta / U_{trans}$. On the other hand, the deflection in a system like Fig. 2 is a complicated function $f(n)$ of the adimensional number $n = \omega a/U_d$, where ω is the angular velocity of a single tagged particle under the rotating field, and we have the requirement that $\tan \theta = f(n)$.

Using Stokes' law for rotation and translation, in an entirely analogous way to that leading to Eq. (1), if we compare devices working with either principle having the same θ , L and H , and working with tagged particles with the same m, a , we have:

$$\frac{t_{trans}}{t_{rot}} \propto \frac{H}{a \nabla H} \sim \frac{\ell}{a} \quad (2)$$

which is precisely the estimate in the simple example of the rolling particle.

Collective segregation

The second method involves no obstacles, and uses phase-separation under gravity. It may in principle be used to enrich arbitrarily low concentrations of marked particles.

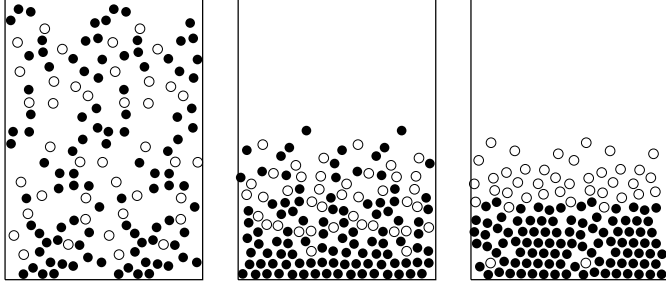


Fig. 3 – Segregation of marked (white) and unmarked (black) particles in a Stokesian dynamics simulation under sedimentation of every particle and forced spinning of the white particles. A bottom wall is simulated by a string of fixed particles (not shown). $n' = 1000$. The time-scales (in $2\pi/\omega$) are, 300 (left), 3000 (center) and 30000 (right). The unit cell has dimensions $L_x = 20a$, $L_y = 200a$ and $L_z = 200a$ (two shown).

Collective segregation results when all the particles (marked and unmarked) sediment under a rotating magnetic field. In such circumstances, isolated particles fall with velocity $U_g = F_s/(6\pi\eta a)$ (where F_s is the sedimentation force) and spin with angular speed ω if they are marked. U_g can be increased by centrifugation, and decreased by matching the density of the particles to that of the fluid.

The basic adimensional parameter of the problem is the ratio of speed of rotation of an isolated tagged particle ω to the speed of sedimentation U_g of an isolated particle $n' = \frac{\omega a}{U_g}$ which can be made large either by increasing the field (and hence ω), or by reducing the sedimentation velocity U_g . *At given geometry and n'* , the time taken for separation depends on ω , and hence can be measured in particle revolutions.

In Fig. 3 we show how separation of tagged (white) and normal (black) particles happens. Initially all the particles are randomly distributed. A sedimentation force F_s acts on every particle in the negative vertical direction while a torque τ , perpendicular to the paper, is applied only to the white particles. The corresponding value of n' is $n' = 1000$ and the times, from left to right are 300, 3000 and 30000 revolutions $2\pi/\omega$. Separation is achieved in a few thousand particle revolutions. Note that there is in principle no limitation on the width of the particle bed, and that the method works with arbitrarily small quantities of marked particles

The reason for this separation is twofold. Cells interact with their neighbours through hydrodynamic interactions. Those that are forced to rotate perturb their near-neighbours, forming a local region in which particles diffuse strongly — *even if the thermal Brownian motion itself is negligible*. Because this diffusion depends on the presence of neighbours, it is weaker in regions of smaller density: this creates a net motion of the rotating particles from regions of high to regions of low particle density — in this example, the surface. This is like having a can of living and dead ants: if the living ones diffuse randomly stepping on the dead bodies, they will eventually reach the surface.

A second, competing effect is the following: the rotating particles also tend to expel their neighbours (see Fig. 4). Hence, they create a low-density region that tends to migrate to the surface through buoyancy.

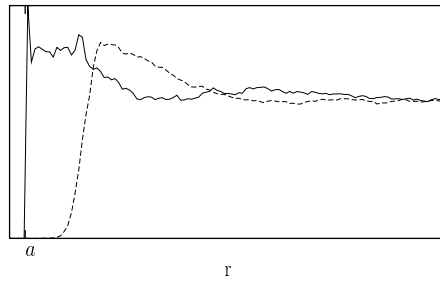


Fig. 4 – Expulsion of neighbours effect. The plots show the distribution function of distances between unmarked particles (full line), and between marked and other particles (dashed line). The curves were calculated with a long-time Stokesian dynamics simulation with 15 free particles and 15 particles under a torque τ , in a cubic cell of side $100a$.

This separation method is strongly reminiscent of the ‘Brazil nut’ phenomenon — the fact that larger particles of a vibrated granular medium tend to go to the surface — since in some cases this can be attributed to the fact that larger particles tend to have stronger diffusivity [19]: here this increased diffusivity is explicitly induced in the marked particles by the rotating field.

Robustness

We have described a separation technique whose basic mechanism is the diffusion generated by the particles that are forced to rotate continuously or alternatively, through the interaction with their closest neighbours or with nearby obstacles. This diffusion is used to sort the particles, by non-magnetic means. The relevant physical principle is robust: the detailed motion the magnetic field induces on the particle, as well as the precise form of the interactions are not of crucial importance. Indeed, we have performed a molecular dynamic simulation with particles interacting through different kinds of forces and confirmed that rotating particles still separate. Furthermore, although we have assumed that the tagged particles rotate around their centers, the basic behaviour would be the same even if only the magnetic tags rotate, since that would in itself create the necessary hydrodynamic interactions.

The method allows to use smaller fields, thus avoiding the magnetic interactions between particles, that may cause an unwanted clustering. It may also be used with larger volumes, and to enrich samples with a very small concentrations of tagged particles. Although we have concentrated mostly on cell separation, the basic mechanism should apply to other kinds of particles.

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